

Comparison of Agarose Gel with ABI

PRISM® 5700

Setup	Results
Tray	Amp Plot
Std Curve	Dissociation
Report	Rn vs Cycles

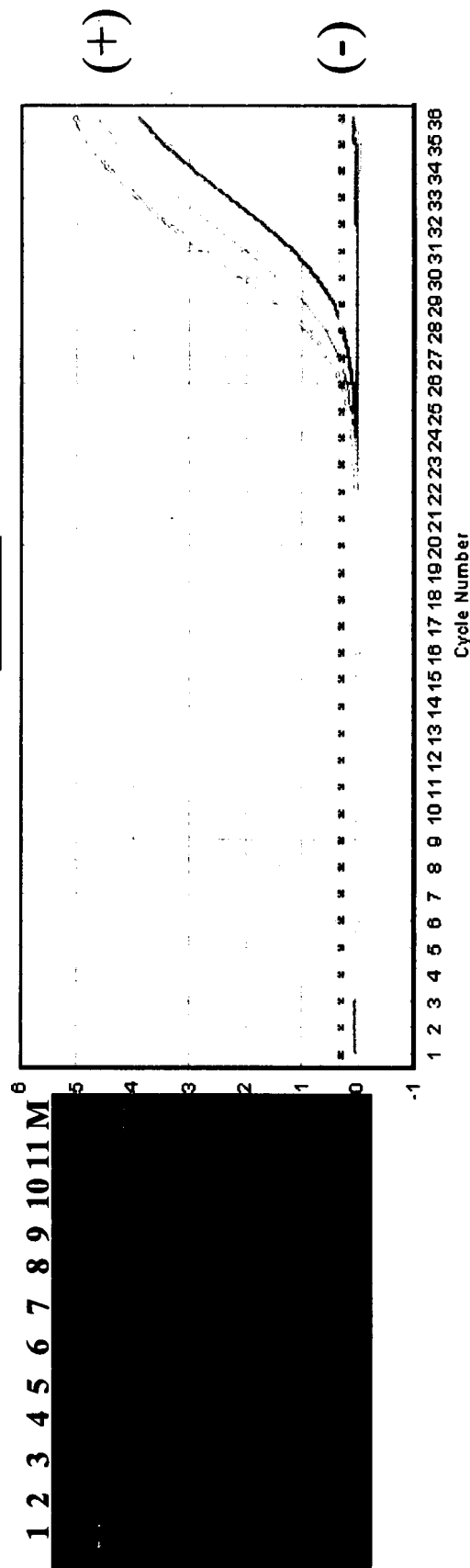


Figure 1. The agarose gel and the ABI PRISM® 5700 show different methods of evaluating PCR results. The gel shows the three positive PCR reactions (Lanes 1, 3 & 9), as well as a control ladder (Lane 12). The agarose gel also shows eight negative PCR reactions. The ABI PRISM® 5700 Sequence Detection System generates an Amplification Plot, which is a measurement of the increase in fluorescence of SYBR green. This increase correlates to an increase of PCR products. The above Amplification Plot shows the four positive reactions (+), and the eight negative reactions (-). The data in the Amplification Plot was collected during the PCR amplification, and the analyzed data was available immediately upon completion of the PCR reactions. The gel shows three positive reactions and not four because the positive control was not loaded, and the control ladder was run in its place.

Low Resolution Typing

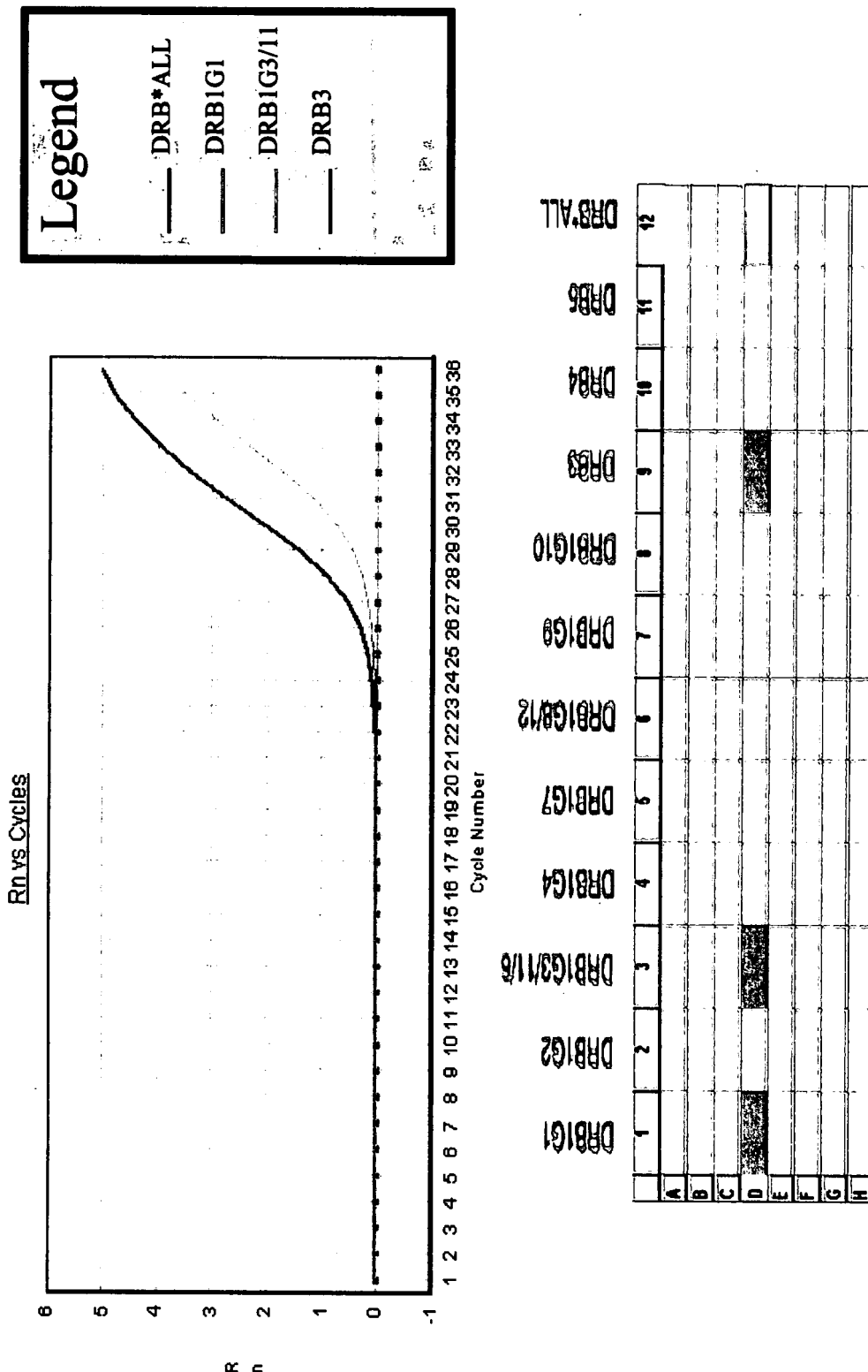


Figure 2. Based on the PCR results, this person is positive for DRB1G1, DRB1G3/11/6 and DRB3. This is an expected combination. This completes the low resolution typing of this individual. These same PCR products were then used for high resolution typing.

High Resolution Typing

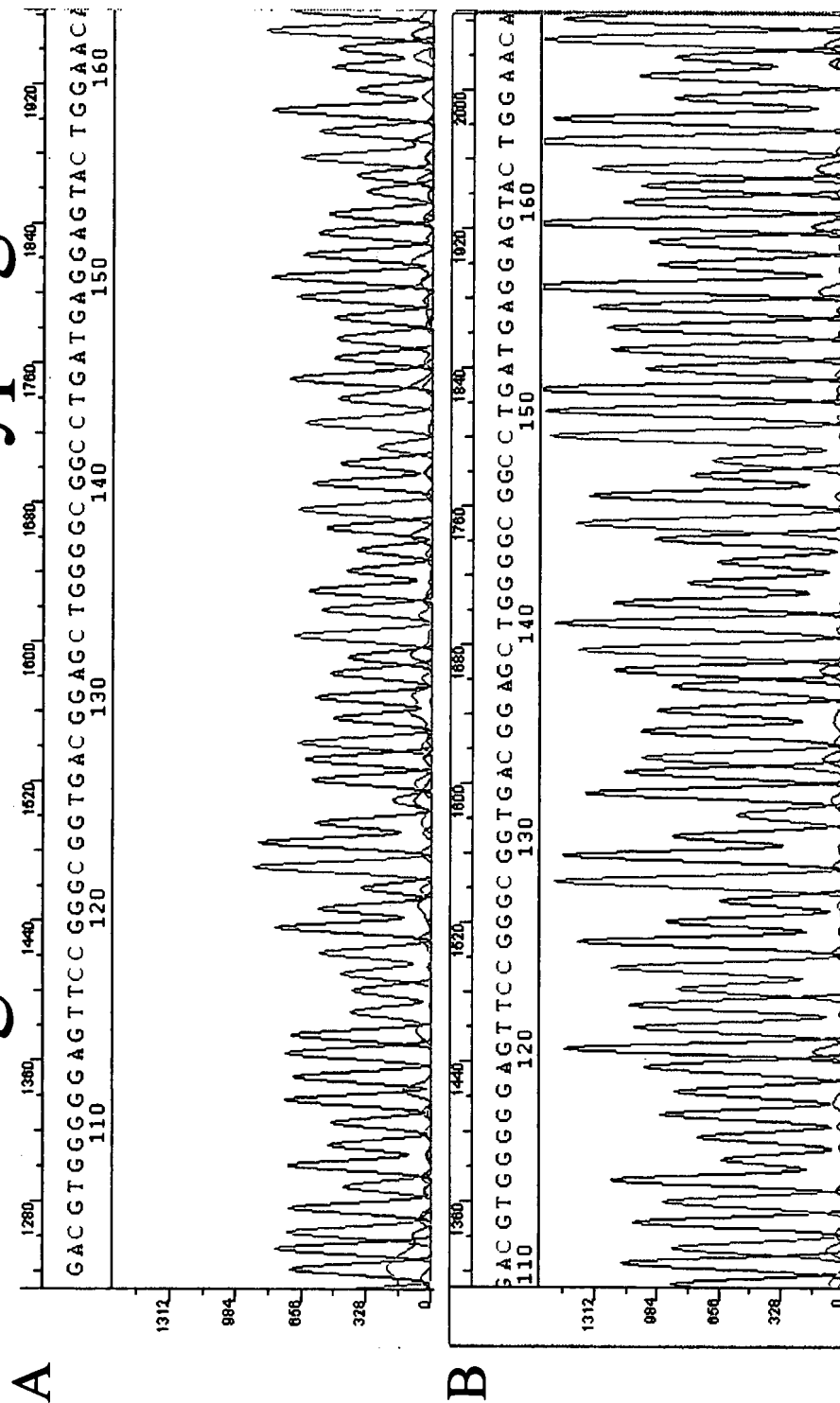


Figure 3. Panel A, shows sequence data from a PCR product produced using the standard HLA protocol. Panel B, shows sequence data from a PCR product generated using the modified SYBR/HLA protocol. Each sample was immediately sequenced after low resolution typing was completed. This comparison of data shows, the addition of SYBR® Green PCR Master Mix had no adverse effect on the sequencing reaction. This data was produced on the ABI PRISM® 3100 Genetic Analyzer.

DF Preliminary Report	
Sample: DF3 Library: DRB1.L155 Preliminary Report: Exact match to: 11011 11011/1105 1105. See Warnings Below. Files : DF3(F.ab1, DF3(R.ab1)	
Warning #3: There are 1 ambiguities at polymorphic positions. Warning #6: There are unexpected base calls at these 1 constant positions:	
1 nucleotide 0 number 1	
A DRB1.L155 consensus M > DF3(F.ab1 A < DF3(R.ab1	
Warnings for file: DF3(F.ab1. #9: The sequence was analyzed with the wrong version of Sequencing Analysis #10: The model 3100 sequencer used is not valid. #11: The peak spacing of 14.84 falls outside of the normal range of 9.0 to	
Warnings for file: DF3(R.ab1. #9: The sequence was analyzed with the wrong version of Sequencing Analysis #10: The model 3100 sequencer used is not valid. #11: The peak spacing of 14.84 falls outside of the normal range of 9.0 to	
Polymorphic Position Report	
4444577788 8899900011 1114567777 7778000111 1112222222 2222222222 2222222222 nucleotide 0268747824 8914728902 3790990134 5899789012 4368012012 34247840 number GGCTTGTGCG TATTAGGTG TCTTGGGTG GGTGCGGCGT CCGCGCGGCT ACCTGTCTC <> DF3 consiR.....H.....> DF3(F.ab >> DF3(R.ab <	
1 DF3(F.ab1 5 2 DF3(R.ab1 3 DF3(F.ab1 6 4 DF3(F.ab1 5 2 DF3(R.ab1 3 DF3(F.ab1 6 4 DF3(F.ab1	0 10 20 30 40 50 60 70 80 90 100 110 120 130 ACGGAGCGGG TCGGTGCT GGAAGATGC ATCTATAACC AAGAGGAGTC CGTGGCGCTC GACAGGACG ACGGAGCGGG TCGGTGCT GGAAGATGC ATCTATAACC AAGAGGAGTC CGTGGCGCTC GACAGGACG ACGGAGCGGG TCGGTGCT GGAAGATGC ATCTATAACC AAGAGGAGTC CGTGGCGCTC GACAGGACG ACGGAGCGGG TCGGTGCT GGAAGATGC ATCTATAACC AAGAGGAGTC CGTGGCGCTC GACAGGACG

DF Final Report	
Sample: DF3 Library: DRB1.L155 Final Report: Exact match to: 11011 11011/1105 1105. See Warnings Below. Files : DF3(F.ab1, DF3(R.ab1)	
Warning #3: There are 1 ambiguities at polymorphic positions. Warnings for file: DF3(F.ab1. #9: The sequence was analyzed with the wrong version of Sequencing Analysis #10: The model 3100 sequencer used is not valid. #11: The peak spacing of 14.84 falls outside of the normal range of 9.0 to	
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1 DF3(F.ab1 5 2 DF3(R.ab1 3 DF3(F.ab1 6 4 DF3(F.ab1 5 2 DF3(R.ab1 3 DF3(F.ab1 6 4 DF3(F.ab1	0 10 20 30 40 50 60 70 80 90 100 110 120 130 ACGGAGCGGG TCGGTGCT GGAAGATGC ATCTATAACC AAGAGGAGTC CGTGGCGCTC GACAGGACG ACGGAGCGGG TCGGTGCT GGAAGATGC ATCTATAACC AAGAGGAGTC CGTGGCGCTC GACAGGACG ACGGAGCGGG TCGGTGCT GGAAGATGC ATCTATAACC AAGAGGAGTC CGTGGCGCTC GACAGGACG ACGGAGCGGG TCGGTGCT GGAAGATGC ATCTATAACC AAGAGGAGTC CGTGGCGCTC GACAGGACG

Figure 4. This panel shows the completion of the high resolution typing. The sequenced sample data was analyzed by the Applied Biosystems MatchTools™ software to get a Preliminary Report. The data was then edited in Applied Biosystems MT Navigator software, before being resubmitted to the Applied Biosystems MatchTools™ software for a Final Report. This sample was an exact match to 11011, 11011/1105, 1105.